LIBBY ASBESTOS SUPERFUNF SITE
OPERABLE UNIT 3
SURFACE WATER TOXICITY TESTING PROTOCOL

1.0 INTRODUCTION

Surface water in the Rainy Creek drainage contains Libby Amphibole asbestos (LA). The toxicity of this material to fish is not known. This document describes the basic protocol that will be followed to evaluate the toxicity of site waters to fish.

The protocol presented here is based on the protocol developed by Belanger (1985).

2.0 PROTOCOL

Test Waters

All test waters will be stored chilled at 10-12°C prior to use in the tests.

Comment [DW1]: I recommend 0-4C in the dark to minimize algal growth.

Reference Water
The reference water will be moderately hard reconstituted laboratory water, prepared in accord with ASTM xxxx.

Site Waters
Test waters will be provided to the toxicity testing laboratory by EPA. Initial studies will utilize waters collected from one or more onsite locations. Subsequent tests may include preparing a dilution series of an on-site water, or use of laboratory water "spiked" with LA.

Water Chemistry

All site waters will be characterized by EPA by analysis for LA, dissolved and total metals, pH, and hardness. All test waters (including reference water) will be monitored in the laboratory for the following parameters:

Parameter	Frequency
Temperature	Daily
pH	Daily
Dissolved oxygen	Twice per week
Ammonia	Start and end of each static renewal

Comment [DW2]: …until swimup and then before and after each renewal.

Test Species

The test species will be rainbow trout (*Oncorhynchus mykiss*)

Life Stage

The life stage will be newly hatched larvae.

Exposure Conditions

Exposure will be performed using a static renewal protocol in 4-Liter aquaria. There will be 15 larvae per aquarium, with three aquaria per test water (a total of 45 larvae per test water).

Water temperature will be maintained at 12 ±1°C.

Exposure duration will be 6 weeks (42 days).

During the larval stage, water will be changed once every 10 days.
Swim-up is expected to occur on or about day 20 (240 degree-days). After swim-up occurs, water will be changed once every three days.

In order to ensure that fiber settling does not occur, each aquarium will be equipped with an air bubbler placed in the bottom and run continuously. In addition, each aquarium will be equipped with a circulating filter, used without any filter media.

Comment [DW3]: The ammonia measures would be a nice piece of information as a surrogate for potential kidney damage, but I am concerned that the ammonia will build up to toxic levels. Did Brinkman provide any insite here? Do we want to have some ammonia scavenging chips available if ammonia levels start to get too high?

Feeding

No feeding will occur during the larval stage.

After swim-up occurs, fish will be fed freshly-hatched brine shrimp (about 12 hours post hatch) at a rate of 5% per day.

Comment [DW4]: Please clarify this. Presumably this means 5% of fish biomass? How is biomass determined as the fish grow?

Endpoints

Endpoints measured during the study will include a) daily, qualitative observations of behavior, b) daily quantitative data on time to swim-up and mortality c) growth, and d) histological examination of exposed organisms.

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Comment [DW5]: Dead organisms will be analyzed for Histo? Or only live ones at the end of the test? I recommend all organisms get histo. Also, it is important to have a log of observations which will allow us to determine the longest amount of time a fish could have been dead to help distinguish rotting from histo abnormalities.

Behavior
All aquaria will be observed daily for indications of differences in behavior between control fish and fish exposed to site waters. This may include, for example, differences in the frequency and duration of swimming events of the larvae, swimming and feeding behavior of the fry, etc.

These observations will be recorded using the behavioral observation log sheet provided as Attachment 1.

Mortality
Observations on mortality will be recorded daily using the form provided as Attachment 2.

Growth
No measures of growth will be performed during the larval stage. After swim-up, measures of growth will include length and mass of the fish. Data on growth will be recorded using the form provided as Attachment 3.

Histopathology

All fish that die during the study and all fish alive at the end of the study will be preserved for histological examination by being placed into 0.1 M phosphate buffered solution containing 5% glutaraldehyde, 3% formalin, and 0.25% picric acid.

Subsequent processing of the tissue will be performed at the histopathology laboratory.

Comment [DW6]: It is important to have a log of observations which will allow us to determine the longest amount of time a fish could have been dead to help distinguish rotting from histo abnormalities. What is a reasonable time frame for observations 2x daily? OH, I NOW SEE THE DATA SHEETS.

Comment [DW7]: I anticipate that we will have to modify this. I don't believe this is the preferred fixative for most fish pathologists. I have attached an SOP that is from the FWS Fish Health Center in Bozeman. I think, for obvious reasons they have phased out the use of picric acid.

REFERENCES

Belanger, S. E. 1985. Functional and pathological Responses of Selected Aquatic Organisms to Chrysotile Asbestos. Doctoral Dissertation approved by Virginia Polytechnic Institute and State University, September 1985.

ATTACHMENT 1. BEHAVIORAL LOG

Day	Observations
1	
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ATTACHMENT 2. MORTALITY LOG

WATER SAMPLE

Day	Aquarium 1			Aquarium 2			Aquarium 3		
	# fish alive at start of day	# fish died during the day	Notes	# fish alive at start of day	# fish died during the day	Notes	# fish alive at start of day	# fish died during the day	Notes
1									
2									
3									
4									
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ATTACHMENT 3. GROWTH LOG

Fish	Aquarium 1			Aquarium 2			Aquarium 3		
	Day of death	Length (mm)	Weight (mg)	Day of death	Length (mm)	Weight (mg)	Day of death	Length (mm)	Weight (mg)
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